



RESEARCH ARTICLE

Impacts of eco-friendly treatments on managing *Alternaria* blight disease and enhancing yield attributes of mustard

Muhammed Ali Hossain^{1*}, Nadira Sultana^{1†}, Fatema Tuz Zohura^{1,2}, Md Atikur Rahman¹, Zahidul Islam¹, Anamika Paul¹ & Md Ibrahim Khalil³

¹Plant Microbe Interaction Laboratory, Department of Plant Pathology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

²Department of Agriculture, Gopalganj Science and Technology University, Gopalganj 8100, Bangladesh

³Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh 2202, Bangladesh

*Correspondence email - alihossain.ppath@bau.edu.bd

† Both authors contributed equally to this work

Received: 19 January 2026; Accepted: 08 June 2026; Available online: Version 1.0: 07 August 2026

Cite this article: Muhammed AH, Nadira S, Fatema TZ, Md Atikur R, Zahidul I, Anamika P, Md Ibrahim K. Impacts of eco-friendly treatments on managing *Alternaria* blight disease and enhancing yield attributes of mustard. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.13730>

Abstract

The antifungal effects of some botanical extracts and the antagonistic effects of *Trichoderma asperellum* isolates against *Alternaria brassicicola*, the causative agent of *Alternaria* blight of mustard was evaluated in this study on November 2021 to February 2022 at Bangladesh Agricultural University, Mymensingh. A total of 18 plant extracts and 18 *Trichoderma* isolates were used to evaluate their effectiveness in inhibiting *A. brassicicola* mycelial growth. The highest mycelial growth inhibition (93.59 %) was observed with cumin seed and clove seed extracts. Five *T. asperellum* isolates exhibited 84.13 % growth inhibition of *A. brassicicola* in *in vitro* dual culture assays. Following these promising findings, field trials were conducted using the best two botanicals at two different concentrations (1:10 and 1:20) and formulated products of two *T. asperellum* isolates (BDPMIL-TAN51 and BDPMIL-TAN41) to evaluate their impact on disease incidence and severity of *Alternaria* blight in the field condition. In field conditions, plants treated with *T. asperellum* bioformulation demonstrated superior results compared to those treated with botanicals, exhibiting minimal disease incidence and severity (19 % and 18.33 % respectively), while water control plants recorded the highest disease incidence and severity (38 % and 78.33 %) at 54 days after sowing (DAS). *Trichoderma asperellum* isolate PMIL-TAN51 bioformulation-treated plants displayed the lowest pod infection rate (20 %), whereas control plants presented the highest pod infection rate (66.63 %). Additionally, plots sprayed with *Trichoderma* bioformulation-treated plants showed better yield-contributing parameters and overall yield compared to those treated with botanicals. According to the results of field evaluation, *T. asperellum*, as a biocontrol agent, may help in managing *Alternaria* blight of mustard disease in Bangladesh.

Keywords: *Alternaria* blight; bioformulation; botanicals; eco-friendly management; mycelial growth inhibition; *Trichoderma asperellum*

Introduction

Mustard (*Brassica* spp.), a member of the Brassicaceae family, is a significant rabi oilseed crop in Bangladesh and a preferred source of edible oil for one-third of the global population. Originating from the Mediterranean region, mustard holds diverse agronomic value, serving as fodder, green manure, fast-growing salads, condiments and edible oilseeds due to its high protein and oil content (1). Mustard is grown in 53 countries across six continents. In 2023, Bangladesh significantly increased its mustard production by expanding its farming area by 40 % (to 812000 hectares). This resulted in a massive harvest of 1.152 million tonnes of seeds (2). Mustard, with its oil content ranging from 40–44 %, protein content of 25 % and nitrogen (N) content of 6.4 %, yields oilcake as a by-product, which serves as a nutritious feed for cattle and fish, as well as an organic fertiliser. Additionally, the young leaves of mustard

are consumed fresh as a salad, possessing therapeutic benefits such as blood purification and being rich in vitamins A, C and E (3–5). Glucosinolates found in mustard have pesticidal properties, which are utilised as fumigants and soil organic matter content, thereby promoting environmentally friendly and cost-effective agricultural practices (6). Mustard cultivation significantly contributes to the income of small and marginal farmers; nevertheless, its production encounters challenges from various biotic and abiotic factors worldwide, with diseases posing a significant threat. In Bangladesh, mustard is susceptible to 14 diseases, among which leaf blight caused by *Alternaria brassicae*/*Alternaria brassicicola* stands out as one of the most devastating, leading to circular spots on leaves and pods, thereby affecting seed quality, size, discolouration and oil content by impeding photosynthesis (7, 8). This fungal infection poses a serious threat to mustard crops, leading to a potential decrease of up to 70 % in

rapeseed yields and 35–40 % in Indian mustard yields, resulting in damage to crop development at every phase and a notable reduction in harvest quality (9). The disease significantly impacts yellow sarson (46–47 %) and mustard (35–38 %), causing substantial losses by disrupting their photosynthetic capabilities and thus becoming one of the most-destructive fungal diseases of Brassicaceae in Bangladesh (10). Currently, no commercially grown mustard cultivars in Bangladesh exhibit resistance to *Alternaria* leaf blight disease, except for BINA Sharisha-8, which shows borderline resistance against *A. brassicicola* (10). Developing resistant varieties is challenging, expensive and time-consuming; hence, adopting eco-friendly management approaches, such as the use of biological agents and botanical extracts, is deemed the most effective strategy for long-term disease control and for preventing environmental damage caused by chemical fungicides (11–13). Several studies have investigated and published eco-friendly management techniques for controlling *Alternaria* blight disease in mustard fields, with bioagents like *Trichoderma* spp. and *Bacillus* spp., along with plant extracts, proving to be the most efficient and cost-effective methods (14, 15). Garlic bulb extract and neem leaf extract have displayed the best results in controlling *A. brassicae* (16, 17), while *Trichoderma* species have demonstrated efficacy in seed treatment and foliar spray applications against *Alternaria* blight (12, 18, 19). Considering the above circumstances regarding this disease, the present study aims to evaluate the effectiveness of specific eco-friendly treatments in controlling *Alternaria* leaf blight disease in mustard fields and their effects on growth and yield parameters.

Materials and Methods

In vitro evaluation of different botanical extracts and *Trichoderma* spp. against *Alternaria brassicicola*

Preparation of *Alternaria* isolates for *in vitro* evaluation

The *in vitro* experiment was carried out at the Plant Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, using a completely randomised design (CRD) with 3 replications per treatment, along with a control for comparison. *Alternaria* blight disease causing organism, *A. brassicicola* was isolated from infected mustard seeds obtained from farmers' fields using the standard blotter method (20). The fungus grew out of seeds in moist blotter within 3–4 days of incubation. Deep brown to dark ashy fungal structures appeared on seeds. Each seed exhibiting such structures was observed under a stereo-binocular microscope. Preliminary observations confirmed that the fungal growth belonged to the genus *Alternaria*. Upon transferring to potato dextrose agar (PDA) plates, colonies with dark coloured top and back appeared. Morphological characteristics were examined for species confirmation and a pure culture was obtained through sequential subculturing of the fungal isolate 3–4 times, following the procedure outlined by previous studies (10, 21).

Screening of different botanical extracts against *Alternaria brassicicola*

The antifungal efficacy of 18 plant extracts against *A. brassicicola* was examined in this experiment (Table 1). Additionally, chemical fungicide, Rovral 50 WP (Iprodione) and water was used as a control were included for comparison. The antifungal activity of the plant extracts was assessed, where the botanical extracts were prepared at a ratio of 1:5 (20 g of fresh plant samples blended in 100 mL water) and then mixed with 100 mL of double strength PDA to make a PDA media containing plant extract at a ratio of 1:10 (22). The PDA medium supplemented with plant extract, was sterilised and poured into Petri dishes to prepare PDA plates. A 0.3 % (w/v) solution of Rovral 50 WP was prepared by dissolving 3 g of the fungicide in 1000 mL of distilled water.

Table 1. List of botanicals with their specifications used *in vitro* experiment

Treatments	Scientific name/ chemical name	Type	Plant parts used	Active ingredient
T ₁ : Garlic	<i>Allium sativum</i> L.	Botanical	Clove	Allicin (23)
T ₂ : Black cumin	<i>Nigella sativa</i> L.	Botanical	Seed	Thymoquinone, thymohydroquinone (24)
T ₃ : Onion	<i>Allium cepa</i> L.	Botanical	Clove	Ergoloid, galantamine (25)
T ₄ : Cumin seed	<i>Cuminum cyminum</i> L.	Botanical	Seed	Terpenes, flavonoids (26)
T ₅ : Fenugreek	<i>Trigonella foenum-graecum</i> L.	Botanical	Seed	4-Hydroxyisoleucine (27)
T ₆ : Mahogany	<i>Swietenia macrophylla</i> King.	Botanical	Seed	3B-acetoxy-mexicanolide, 6-acetyl-swietenine (28)
T ₇ : Black pepper	<i>Piper nigrum</i> L.	Botanical	Seed	Piperine (29)
T ₈ : Clove seed	<i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry	Botanical	Seed	Eugenol (30)
T ₉ : Neem	<i>Azadirachta indica</i> A.Juss.	Botanical	Leaf	Azadirachtin (31)
T ₁₀ : Allamonda	<i>Allamanda cathartica</i> L.	Botanical	Leaf	Hexanoic acid, octanoic acid (32)
T ₁₁ : Tulsi	<i>Ocimum tenuiflorum</i> L.	Botanical	Leaf	Methyl chavicol, linalool (33)
T ₁₂ : Mehendi	<i>Lawsonia inermis</i> L.	Botanical	Leaf	lawsone (2-hydroxy-1,4-naphthoquinone) (34)
T ₁₃ : Bishkatali	<i>Polygonum hydropiper</i> L.	Botanical	Leaf	Sesquiterpene dialdehydes polygodial (35)
T ₁₄ : Acasia	<i>Acacia auriculiformis</i> A.Cunn. ex Benth.	Botanical	Leaf	1,1-diphenyl-2-picrylhydrazyl (DPPH), Teracacidin (36)
T ₁₅ : Curry leaves	<i>Murraya koenigii</i> (L.) Spreng.	Botanical	Leaf	Mahanine (37)
T ₁₆ : Pepper mint	<i>Mentha piperita</i> L.	Botanical	Leaf	Menthol (38)
T ₁₇ : Nishinda	<i>Vitex negundo</i> L.	Botanical	Leaf	Hexadecanoic acid, caryophyllene oxide (39)
T ₁₈ : Lantana	<i>Lantana camara</i> L.	Botanical	Leaf	β-caryophyllene, icterogenin (triterpenes) (40)
T ₁₉ : Rovral 50 WP (0.2 %)	-	Chemical	-	Iprodione 50 %
T ₂₀ : Control	without plant extract	Only PDA	-	-

PDA: potato dextrose agar.

Screening of different *Trichoderma* isolates against *Alternaria brassicicola*

This study examined the antagonistic activity of 18 *Trichoderma* isolates collected from Plant Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University against *A. brassicicola* pathogen causing *Alternaria* blight disease in mustard-rape seed. The effects of *Trichoderma* on the development of *Alternaria* sp. were assessed using dual-culture procedures (41). The antagonistic effect of *Trichoderma* on *Alternaria* sp. was measured where *Trichoderma* and *Alternaria* cultures were placed at equal distances from the periphery of the same Petri dishes containing PDA. The plates were incubated at 25 °C for 10 days at a 12/12 hr dark/light condition. Both treatment and control plates were maintained with 3 replications. At intervals of 3, 5 and 7 days, the colony diameter of the targeted fungal pathogen was measured in centimeters and the percentage inhibition of the pathogen's typical radial mycelial growth was calculated (42).

Measurements of mycelial growth

Mycelial growth of *A. brassicicola* was measured by calculating the average of two perpendicular colony diameters. Measuring the average of two diameters at right angles to one another. The formula given below was used to determine the percentage of growth inhibition caused by botanicals extracts and *Trichoderma* (43).

$$\text{Growth inhibition (\%)} = \frac{C-T}{C} \times 100 \quad (\text{Eq. 1})$$

Where, C = mean mycelial growth (radial) of the pathogen in the control plate, T = mean mycelial growth (radial) of the pathogen in the treated plate.

Field evaluation of botanical extracts and bio-control agents against *Alternaria brassicicola*

The experiment was conducted at the field laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and Plant Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. BARI Sarisha 4 (Tori-7), one of the high-yielding mustard varieties in Bangladesh, was used for this field experiment. BARI Sharisha 4 is also susceptible to *Alternaria* disease (10). A total of 24 plots were prepared for 8 treatments (Table 2), with 3 replications maintained. Seeds were sown on 18 November 2021 in each experimental plot (each plot size was 1 m²

row to row spacing 30 cm and plant to plant spacing 5 cm) following the standard planting method, the recommended dose of fertilisers was applied and proper irrigation and weeding were maintained (44). Botanical extracts and *Trichoderma*-based bioformulations were selected based on the results of *in vitro* inhibition.

Application of botanical extracts and *Trichoderma* bioformulations

Eight treatments were used to evaluate the disease incidence and severity of *Alternaria* blight of mustard, along with various yield-contributing parameters. Based on the effectiveness observed *in vitro* assay, two botanicals (cumin and clove seed extracts) at two concentrations (1:10, 1:20) and two *T. asperellum*-based bioformulations (BDPMIL-TAN51, BDPMIL-TAN41) were selected. Chemical fungicide Rovral 50 WP (Iprodione) was used as a positive control and water as a negative control. The 1:10 and 1:20 concentrations of cumin and clove seed extracts were made by adding the necessary amount of water to a 1 : 5 stock solution of cumin and clove seed extracts. The Plant Microbe Interaction Lab, Department of Plant Pathology at Bangladesh Agricultural University in Bangladesh provided the formulated *T. asperellum* bio-agent (BDPMIL-TAN51, BDPMIL-TAN41), 3 g of each formulation was mixed with distilled water to a final volume of 1000 mL using a volumetric flask. For the chemical control, 1.5 g of Rovral 50 WP was dissolved in 500 mL of distilled water. Botanical extracts, *Trichoderma*-based bio-formulations and chemical fungicide were foliar sprayed three times at 7 days intervals, starting from 33 days after sowing (DAS). Disease incidence and severity of *Alternaria* blight were measured at seven days after each application at 40, 47 and 54 DAS.

Disease observation

For each plot, five plants were considered randomly to find out the disease incidence by using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100 \quad (\text{Eq.2})$$

Disease severity of naturally infected BARI Sarisha 4 (Tori-7) plants in treated and non-treated plots was assessed by a disease severity scale (45) (Table 3).

Assessment of percent pod infection

For each of the plot, five plants were considered randomly to find out the percentage pod infection by using the following formula (46):

Table 2. Treatments used in the field experiment with specifications

Treatments	Type	Active ingredient	Concentration
T ₁ : Cumin seed (<i>C. cyminum</i>)	Botanical	Terpenes, flavonoids (26)	1 : 10
T ₂ : Cumin seed (<i>C. cyminum</i>)	Botanical	Terpenes, flavonoids (26)	1 : 20
T ₃ : Clove seed (<i>S. aromaticum</i>)	Botanical	Eugenol, flavonoids (30)	1 : 10
T ₄ : Clove seed (<i>S. aromaticum</i>)	Botanical	Eugenol, flavonoids (30)	1 : 20
T ₅ : Formulation of <i>T. asperellum</i> isolate BDPMIL-TAN51	Biological	<i>Trichoderma asperellum</i>	3 g/1000 mL
T ₆ : Formulation of <i>T. asperellum</i> isolate BDPMIL-TAN41	Biological	<i>T. asperellum</i>	3 g/1000 mL
T ₇ : Rovral 50 WP	Chemical	Iprodione	0.3 %
T ₈ : Control	Only water	-	-

Table 3. Disease severity scale for *Alternaria* leaf blight

Rating scale	Symptoms produced on leaves are categorised
1	No spots and no chlorosis on the leaf
2	A few pinpoint spots but no chlorosis
3	Some spots but no large lesions and no chlorosis
4	Some spots with a few lesions surrounded by light chlorosis
5-9	Increasing number and size of lesions and chlorosis on the leaf
10	Lesions with chlorosis on more than 90 % of the leaf

$$\text{Pod infection (\%)} = \frac{\text{No. of infected pods per plant}}{\text{Total no. of pods per plant}} \times 100 \quad (\text{Eq.3})$$

Data collection for the yield and yield contributing parameters

Number of seeds per plant, no. of infected seeds per plant, no. of seeds per pod, weight of diseased seeds per plant, weight of healthy seeds per plant, weight of 1000-seeds and yield per hectare collected during the maturity stage of the plant. The data on different parameters were statistically analysed using analysis of variance (ANOVA) technique to find out the level of significance (47). The treatment means were compared by Duncan's multiple range test (DMRT) at a 5 % level of significance. The data collected were analysed using the SAS (University Edition version 3.71 basic edition) statistical package.

Results

In vitro evaluation of different botanical extracts on radial mycelial growth and percent growth inhibition of *Alternaria brassicicola*

Botanical extracts showed a highly significant effect on radial mycelial growth and percent growth inhibition of *Alternaria brassicicola* in case of all treatments (DMRT, $p \leq 0.05$; LSD = 0.3701 at 10 days after inoculation (DAI)). Under controlled conditions, cumin seed extracts (T_4) and clove seed extracts (T_8) greatly inhibited the pathogen's mycelial development, reducing the growth to just 0.5 cm at 10 DAI (93.59 % inhibition) over the untreated control (T_0) 7.8 cm, placing them statistically same with Rovral 50 WP (T_{19} ; 0.5 cm, 93.59 %) and together forming the highest-performing group (shared letter m, DMRT). This near-complete suppression demonstrates that these two botanicals can match synthetic fungicide performance in *in vitro* at a 1 : 10 (plant parts: water + PDA) dilution. Mycelial growth of *A. brassicicola* in different treatments varied substantially, with highest mycelial growth recorded under control and the least

mycelial growth recorded in PDA medium supplemented with cumin and clove seed extracts, followed by almond leaf extract (1.47 cm at 10 DAI) and forming a statistically distinct group (letter l) from both the cumin/clove extracts and the mid-range treatments. In contrast, mahogany seed extract (T_6) showed the weakest inhibitory activity (16.28 %; 6.53 cm), significantly worse than most treatments (letter b) and approaching the performance of the untreated control (letter a). The wide range of inhibition values (16.28–93.59 %) across the 18 extracts confirms that biocontrol potential is highly species-specific and cannot be generalised across botanicals (Table 4 and Fig. 1).

In vitro evaluation of *Trichoderma* spp. on radial mycelial growth of *Alternaria brassicicola*

A total of 18 *Trichoderma* isolates were used in this study to know the effect of *Trichoderma* spp. in suppressing the radial mycelial growth of *A. brassicicola* in dual culture assays up to 7 days. Dual-culture assays demonstrate a substantial and statistically significant variation in the antagonistic capacity of 18 *Trichoderma* isolates against *A. brassicicola* (LSD_{0.05} = 0.2144 at 7 DAI). Five isolates (HI, N51, R1, N41 and R50) out of eighteen were found to restrict the growth of the fungus after 3 days of inoculation in dual cultures and within 5 days mycelial growth of *A. brassicicola* was totally overlapped by the mycelial growth of different *Trichoderma* isolates and the mycelial growth of *A. brassicicola* was entirely inhibited due to the overgrowth of *Trichoderma* isolates. These five isolates achieved the maximum recorded suppression of 84.13 % at 7 DAI, reducing pathogen growth to just 1.0 cm against a control plate value of 6.3 cm and forming a statistically superior group (letter h, 7 DAI) other than all remaining isolates. Radial growth of *A. brassicicola* was reduced by 46.03 to 84.13 % in dual culture assays at 7 DAI (Table 5). Isolates SIA and I1 were the weakest performers, achieving only 47.62 % and 46.03 % inhibition respectively at 7 DAI (letter a, highest growth values), which was less than half the suppression achieved by the top isolates. The five *Trichoderma* isolates were identified as *Trichoderma asperellum* by sequencing of internal transcribed spacer (ITS) region (data not published), indicated a rapid and aggressive mode of antagonism (Table 5 and Fig. 2).

Table 4. *In vitro* evaluation of different plant extracts on radial mycelial growth of *Alternaria brassicicola*

Treatments	Mycelial growth (cm) 5 DAI ^a	Mycelial growth (cm) 10 DAI	Percentage growth inhibition over control (5 DAI)	Percentage growth inhibition over control (10 DAI)
T ₁ - Garlic clove extract (1 : 10)	3.4de	5.5f	15	29.49
T ₂ - Black cumin seed extract (1 : 10)	1.97h	3.8h	50.75	51.28
T ₃ - Onion clove extract (1 : 10)	3.43de	6.1cd	14.25	21.79
T ₄ - Cumin seed extract (1 : 10)	0.5k	0.5m	87.5	93.59
T ₅ - Fenugreek seed extract (1 : 10)	3.78bc	5.9de	5.5	24.39
T ₆ - Mahogany seed extract (1 : 10)	3.1f	6.53b	22.5	16.28
T ₇ - Black pepper seed extract (1 : 10)	2.53g	4.63g	36.75	40.64
T ₈ - Clove seed extract (1 : 10)	0.5k	0.5m	87.5	93.59
T ₉ - Neem leaf extract (1 : 10)	3.67cd	6.4bc	8.25	17.95
T ₁₀ - Almond leaf extract (1 : 10)	0.97j	1.47l	75.75	81.15
T ₁₁ - Tulsi leaf extract (1 : 10)	3.23ef	6.4bc	19.25	17.95
T ₁₂ - Mehendi leaf extract (1 : 10)	1.57i	3.2ij	60.75	58.97
T ₁₃ - Bishkatali leaf extract (1 : 10)	1j	2.03k	75	73.97
T ₁₄ - Acasia leaf extract (1 : 10)	4.1a	5.68ef	00	27.179
T ₁₅ - Curry leaf extract (1 : 10)	1.97h	3.43hi	50.75	56.03
T ₁₆ - Pepper mint leaf extract (1 : 10)	3.97ab	5.97de	0.75	23.46
T ₁₇ - Nishinda leaf extract (1 : 10)	1.9h	3.05j	52.5	60.90
T ₁₈ - Lantana leaf extract (1 : 10)	2.7g	4.43g	32.5	43.21
T ₁₉ - Rovral 2 % (50 WP)	0.5k	0.5m	87.5	93.59
T ₀ - Control	4ab	7.8a	-	-
LSD ^b	0.2825	0.3701	-	-

^aDAI: days after inoculation; data represent the means of 4 replications; ^bLSD (0.05): least significant difference. In a column, means followed by the same letter(s) are statistically similar at 5 % level by DMRT.

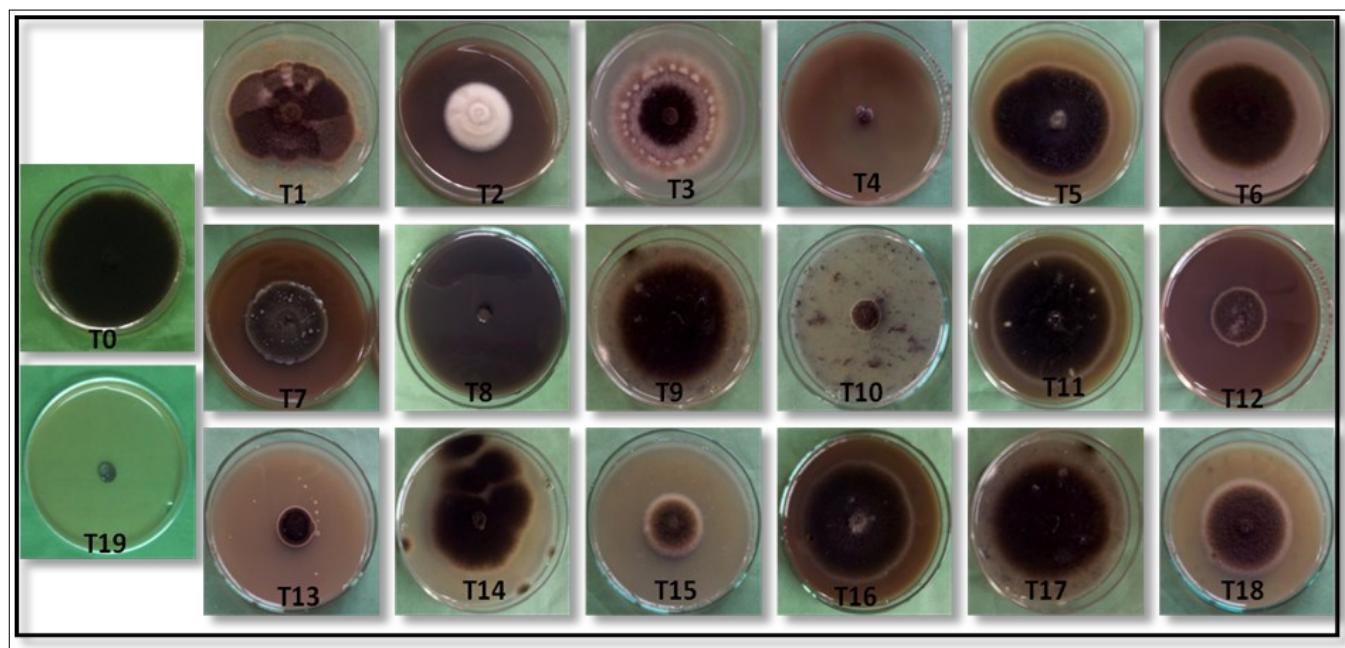


Fig. 1. *In vitro* mycelial growth suppression of *Alternaria brassicicola* with potato dextrose agar media supplemented with different plant extracts (1:10) after 10 days of inoculation.

DAI: Days after inoculation

Table 5. *In vitro* mycelial growth suppression of *Alternaria brassicicola* with *Trichoderma* spp.

Trichoderma isolate	Radial mycelial growth of <i>A. brassicicola</i> on control plate (cm)			Radial mycelial growth of <i>A. brassicicola</i> (cm) on dual culture plate			Percentage growth inhibition of <i>A. brassicicola</i> on dual culture plate over control plate		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI ^a
Isolate HI	3.5a	4.5a	6.3a	1.0j	1.0g	1.0h	71.43	77.78	84.13
Isolate N51	3.5a	4.5a	6.3a	1.0j	1.0g	1.0h	71.43	77.78	84.13
Isolate J1	3.5a	4.5a	6.3a	2.2c	2.6bc	2.7b	37.14	42.22	57.14
Isolate R1	3.5a	4.5a	6.3a	1.0j	1.0g	1.0h	71.43	77.78	84.13
Isolate N41	3.5a	4.5a	6.3a	1.0j	1.0g	1.0h	71.43	77.78	84.13
Isolate SIA	3.5a	4.5a	6.3a	2.8a	3.0a	3.3a	20.00	33.33	47.62
Isolate R50	3.5a	4.5a	6.3a	1.0j	1.0g	1.0h	71.43	77.78	84.13
Isolate B2	3.5a	4.5a	6.3a	1.2i	1.4f	1.5g	65.71	68.89	76.19
Isolate Ku1	3.5a	4.5a	6.3a	2.0de	2.3d	2.5bc	42.86	48.89	60.32
Isolate B1	3.5a	4.5a	6.3a	1.8fg	2.0e	2.1f	48.57	55.56	66.67
Isolate C1	3.5a	4.5a	6.3a	2.2c	2.3d	2.4de	37.14	48.89	61.90
Isolate T2	3.5a	4.5a	6.3a	2.1cd	2.4cd	2.6bc	40.00	46.67	58.73
Isolate S1	3.5a	4.5a	6.3a	1.9ef	2.3d	2.5bc	45.71	48.89	60.32
Isolate N3	3.5a	4.5a	6.3a	1.7g	2.0e	2.2ef	51.43	55.56	65.08
Isolate S2	3.5a	4.5a	6.3a	2.0de	2.3d	2.6bc	42.86	48.89	58.73
Isolate N91	3.5a	4.5a	6.3a	1.5h	1.9e	2.3ef	57.14	57.78	65.08
Isolate N61	3.5a	4.5a	6.3a	2.1cd	2.4cd	2.7b	40.00	46.67	57.14
Isolate I1	3.5a	4.5a	6.3a	2.4b	2.7b	3.4a	31.43	40.00	46.03
LSD (0.05) ^b	0.4876	0.5436	0.4362	0.1448	0.2038	0.2144	-	-	-

^aDAI: Days after inoculation; data represents the means of 4 replications; ^bLSD (0.05): least significant difference. In a column, means followed by same letter(s) are statistically similar at 5 % level by DMRT.

Effect of botanicals and formulated *Trichoderma asperellum* on diseases incidence and disease severity of *Alternaria* blight of mustard in the field

Effects of different treatments on disease incidence and severity were statistically significant at all three assessment points, with significance levels increasing over the season, from $p \leq 0.05$ at 47 DAS to $p \leq 0.001$ at both 40 and 54 DAS for incidence and $p \leq 0.01$ at 40 and 47 DAS intensifying to $p \leq 0.001$ at 54 DAS for severity, which indicate the differences progressed among different treatments over time reflecting (Table 6). The effect of two botanicals (cumin seed and clove seed) and two bio-agents, *T. asperellum* based bioformulation PMIL-TAN51 and PMIL-TAN41 on *Alternaria* blight of mustard in the field was studied at 40 DAS, 47 DAS and 54 DAS i.e., 7 days after each treatment application. Plants treated with *Trichoderma* performed better than other eco-

friendly management methods in reducing *Alternaria* blight incidence and severity. However, at 54 DAS, the minimum percentage of disease incidence of treated plants was 19 % in case of T₅ (BDPMIL-TAN51 bioformulation) while the greatest percentage of disease incidence was 38 % in case control (T₀). At 54 DAS, Rovral 50 WP (at 0.3 %) treated plants (positive control) had the lowest proportion of disease incidence (14.00 %). However, in this experiment, the lowest percentage of disease severity in plants treated with formulated product of *T. asperellum* isolate PMIL-TAN51 (T₅) was recorded at 18.33 %, while the highest percentage of disease severity (78.33 %) was observed in plants treated with the control (T₀) at 54 DAS. On the other hand, after 54 DAS, Rovral 50WP (at 0.3 %) treated plants had the lowest percentage of disease severity observed (13.67 %) (Table 6). Critically, T₅ and the positive control Rovral 50WP (T₇: 14 % incidence, 13.67 % severity)

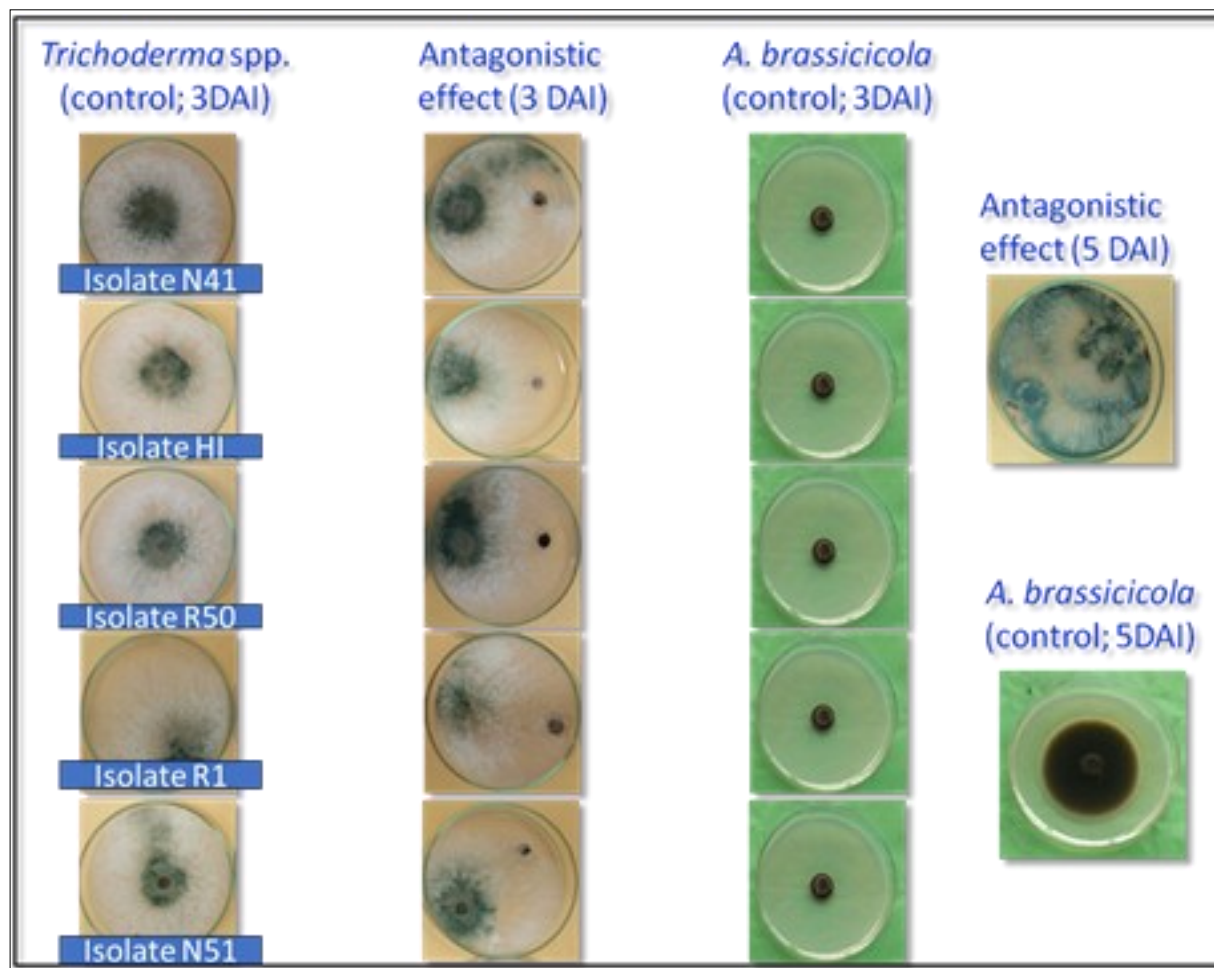


Fig. 2. *In vitro* mycelial growth suppression of *Alternaria brassicicola* with five best performed (N41, HI, R50, R1 & N51) *Trichoderma* spp. isolates after 5 days of inoculation.

Table 6. Effect of plant extracts and bio-agents on disease incidence and severity of *Alternaria* blight disease in the field

Treatments	Disease incidence (%)			Disease severity (%)		
	40 DAS ^a	47 DAS	54 DAS	40 DAS	47 DAS	54 DAS
T ₀ - Control	8.67bc	17ab	38a	15a	21.67a	78.33a
T ₁ - Cumin seed extract (1 : 10)	15ab	17ab	22bc	7ab	12.33ab	75a
T ₂ - Cumin seed extract (1 : 20)	9.67bc	16.67ab	28bc	8.67ab	25.33a	45b
T ₃ - Clove seed extract (1 : 10)	11bc	15.33ab	21.67bc	6ab	11.33ab	43.33b
T ₄ - Clove seed extract (1 : 20)	19a	19.33a	20.67bc	4.67b	12ab	41.66667b
T ₅ - formulation of <i>T. asperellum</i> isolate BDPMIL-TAN51	7.67bc	10.67ab	19bc	4b	5.67b	18.33333cd
T ₆ - formulation of <i>T. asperellum</i> isolate BDPMIL-TAN41	8bc	14ab	19.67bc	10.67ab	17.33ab	38.33333bc
T ₇ - Rovral (0.3 %)	6c	9.67b	14c	2b	4.67b	13.66667d
Level of significance	***	*	***	**	**	***
CV (%)	24.45	22.50	16.06	46	37.26	17.77

In a column, means followed by the same letter(s) are statistically similar at 5 % level by DMRT; ^aDAS: days after sowing; ***: 0.001 level of significance; **: 0.01 level of significance; *: 0.05 level of significance.

shared overlapping or adjacent DMRT groups at 54 DAS for both incidence (letters bc vs c) and severity (letters cd vs d), indicating that their performance was not statistically distinct, that means this bioformulation can be an alternative to chemicals. The PMIL-TAN41 formulation (T₆) also performed well (19.67 % incidence; 38.33 % severity at 54 DAS), though it was statistically inferior to T₅ in severity reduction (letter bc vs cd). Botanical treatments (T₁-T₄) produced intermediate outcomes: clove seed extract at 1 : 20 dilution (T₄) achieved 20.67 % incidence and 41.67 % severity, significantly better than control for severity (letter b), but cumin extract at 1 : 10 (T₁) showed anomalously high early incidence at 40 DAS (letter a, 15 %), suggesting a possible stimulatory or phytotoxic effect at higher concentrations in early growth stages that warrants further investigation.

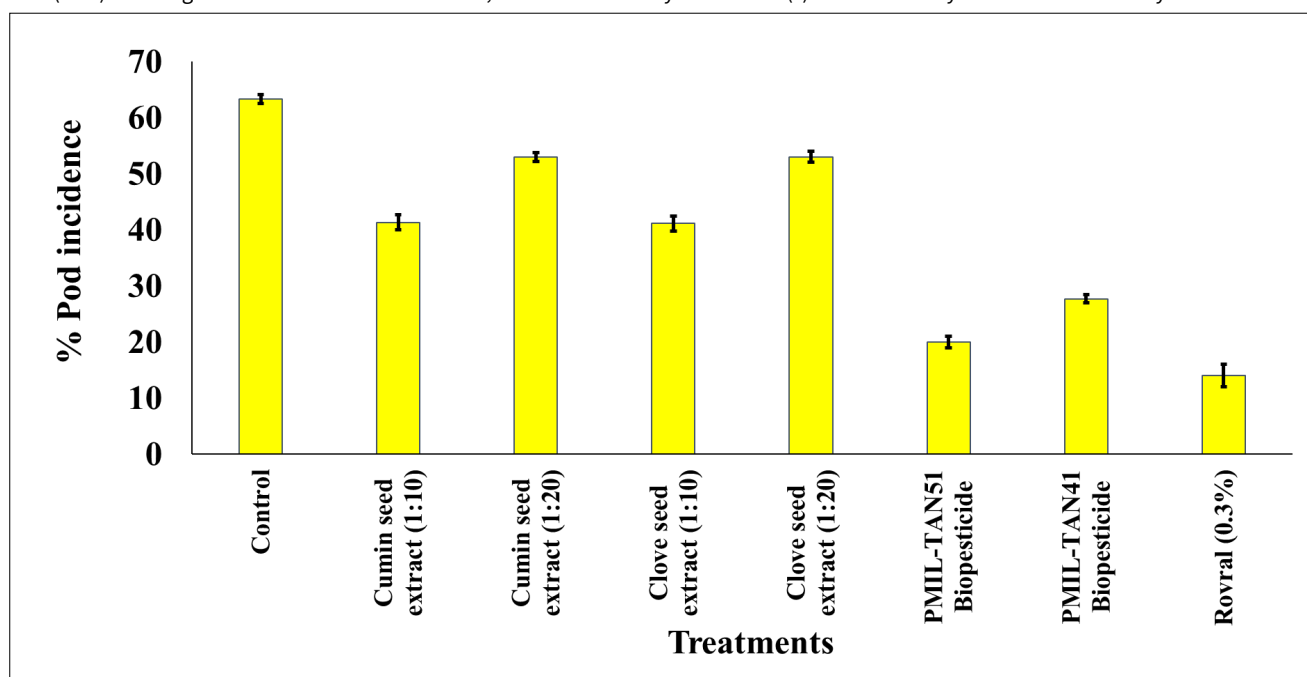
Effect of botanicals and formulated *Trichoderma asperellum* on pod infection for *Alternaria* blight of mustard in the field

The minimum percentage of pod infection (14.00 %) was measured for Rovral 50 WP treated plants (positive control) followed by (20.00 %) in case of plants treated with formulated product of *T. asperellum* isolate PMIL-TAN51 (T₅) and the maximum percentage of pod infection (63.33 %) was recorded in case of control (T₀) plants at 75 DAS; in contrast botanicals showed pod infection rate (40–53 %) near to control treated plants (Table 7 and Fig. 3.). These data indicate a significant and statistically meaningful variation in the antagonistic capacity of different botanicals and formulated *Trichoderma asperellum* on pod infection for *Alternaria* blight of mustard in the field condition (LSD_{0.05} = 5.79 at 75 DAS).

Table 7. Effect of treatments on pod incidence rate in field conditions at 75 days after sowing

Treatments	Pod incidence (%)
T ₀ : Control	64a
T ₁ : Cumin seed extract (1 : 10)	53b
T ₂ : Cumin seed extract (1 : 20)	53b
T ₃ : Clove seed extract (1 : 10)	41c
T ₄ : Clove seed extract (1 : 20)	40c
T ₅ : formulation of <i>T. asperellum</i> isolate BDPMIL-TAN51	28d
T ₆ : formulation of <i>T. asperellum</i> isolate BDPMIL-TAN41	20e
T ₇ : Rovral (0.3 %)	14f
LSD	5.79

^bLSD (0.05): least significant difference. In a column, means followed by same letter(s) are statistically similar at 5 % level by DMRT.

**Fig. 3.** Percent pod infection under different treatments in field conditions at 75 days after sowing.

Effect of eco-friendly treatments on the yield and yield contributing parameters of mustard

All yield and yield contributing attributes were significantly affected by different treatment used in the field experiment, with the majority significant at $p \leq 0.001$ and seed weight per pod significant at $p \leq 0.01$ (Table 6). Among the eco-friendly treatments, the maximum number of seeds/plant (746; letter b) was observed in T₅ (BDPMIL-TAN51 bioformulation) treated plants followed by cumin extract (641.67; letter bc; T₂) treated plants, whereas Rovral 50 WP (0.3 %) treated plants produced 1258 (letter a) seeds/plant, then in the control (395; letter c). The minimum number of infected seeds/plant (11.67; letter b) was observed in T₇ (Rovral 0.3 %) treated plots followed by BDPMIL-TAN51 bioformulation treated plots (12.3; letter b; T₅) and the highest number

of infected seeds/plant (24; letter a) was found in the control plots. The maximum number of seeds/pod (18.33) was observed in T₅ (PMIL-TAN51 bioformulation) treated plots among the eco-friendly treatments, whereas the lowest number of seeds/pod (11.33) was found in the control plots. Diseased seed weight per plant was also minimised in T₅ plots (0.03 g; letter b), statistically equivalent to Rovral 50WP (letter b), while the control recorded 0.047 g (letter ab), though this parameter showed the highest variability across treatments (CV % = 41.78). Healthy seed weight per plant was 76.9 % higher in T₅ plots (2.83 g; letter b) versus control (1.6 g; letter c), directly reflecting the impact of disease reduction on grain-filling efficiency.

However, the weight of 1000 seeds ranged from 2.73 to 4.97 g for T₀ to T₇. In case of the weight of 1000 seeds, T₅ (BDPMIL-TAN51

Table 8. Effect of different eco-friendly management approaches on the yield and yield contributing parameters of mustard

Treatment	Number of seed/plant	Number of infected seed/plant	Number of seed/pod	Weight of diseased seed/plant (g)	Weight of healthy seed/plant (g)	Weight of 1000 seeds (g)	Yield (kg/ha)
T ₀ - Control	395c	24a	11.33b	0.047ab	1.6c	3.52bc	1197.67d
T ₁ - Cumin seed extract (1:10)	475.33c	22a	16ab	0.06ab	1.93bc	4.26ab	1366.67bc
T ₂ - Cumin seed extract (1:20)	641.67bc	15b	17ab	0.04ab	2.5bc	3.4bc	1242cd
T ₃ - Clove seed extract (1:10)	609.33bc	15b	14ab	0.09a	2.29bc	2.73c	1255.67cd
T ₄ - Clove seed extract (1:20)	575.67bc	12.67b	15.67ab	0.04ab	2.33bc	3.73abc	1342bcd
T ₅ - PMIL-TAN51 Biopesticide	746b	12.33b	18.33a	0.03b	2.83b	4.54ab	1442.33b
T ₆ - PMIL-TAN41 Biopesticide	563bc	12.3b	12.67ab	0.06ab	2.267bc	3.96abc	1393.33bc
T ₇ - Rovral (0.3 %)	1258a	11.67b	17.67a	0.03b	4.4a	4.97a	1612a
Level of significance	***	***	**	*	***	***	***
CV (%)	13.49	14.54	13.57	41.78	13.39	11.35	3.95

In a column, means followed by the same letter(s) are statistically similar at 5 % level by DMRT.***: 0.001 level of significance; **: 0.01 level of significance; *: 0.05 level of significance.

bioformulation) treated plants showed maximum weight (4.63 g) while the lowest (2.73 g) weight of 1000 seeds were observed in the plants belonging to control plots. At the crop level, T₅ plots yielded 1442.33 kg/ha (letter b), which was a statistically significant 20.4 % improvement over the control (1197.67 kg/ha; letter d), while Rovral 50WP remained the highest-yielding treatment (1612 kg/ha; letter a), as expected for a chemical standard (Table 8).

Discussion

Mustard, a global condiment, is affected by *Alternaria* blight, causing significant yield losses. Chemical fungicides are the primary method used in Bangladesh to combat *Alternaria* leaf blight, but they pose environmental and human health risks. However, certain plant species have been discovered to contain natural substances in their leaves and bulbs that are toxic to many fungi causing plant diseases (48, 49). This study aims to assess the effectiveness of eco-friendly treatment approaches, specifically plant extracts and *Trichoderma* bioformulations, in controlling *Alternaria* leaf blight in the field.

Plant extracts like cumin and clove seed significantly inhibited the radial mycelial growth of *A. brassicicola*, with 93.59 % inhibition at 10 DAI, whereas mahogany seed extract displayed the lowest percentage of mycelial growth inhibition at 16.28 % compared to the control. Clove extracts demonstrated antifungal activity with minimum inhibitory concentrations (MICs) of 1900, 2300 and 470 ppm against *A. brassicicola* and phenolic compounds present in botanicals could potentially disrupt enzymes involved in spore germination or interfere with amino acids, affecting virulence (50). Moreover, at concentrations of $\geq 750 \mu\text{L/L}$, the essential oil of cumin suppressed post-harvest fungal infections such as *Botrytis cinerea*, *Aspergillus niger* and *Penicillium expansum* (51).

The use of biocontrol strains for the management of plant pathogens has recently been exploited by researchers (52, 53). In this study, the growth of *A. brassicicola* was reduced by 84.13 % in dual culture assays, with *Trichoderma* isolates HI, N51, N41, R1 and R50 showing the highest suppression. *Trichoderma* species can outcompete pathogens like *Macrophomina phaseolina* by inducing hyphal coiling, abnormalities and reduced sclerotial production (54). Some bioagents exhibited static activity and inhibited mycelial growth of *A. brassicae*, with *Trichoderma harzianum* being the most effective, inhibiting up to 65.83 % of the test pathogen (49). They also exhibit the ability to synthesise both diffusible and volatile metabolites, along with volatile and non-volatile antibiotics, giving them a competitive edge in nutrient and spatial competition with pathogens. The antagonistic action of *Trichoderma* spp. is demonstrated *in vitro* by reducing test-fungi growth and fast expansion over the pathogen's colony (55, 56). The incidence of *Alternaria* can be reduced by 25–30 % *in vitro* when chemical fungicides (mancozeb and propiconazole) are applied; nevertheless, *Trichoderma* species alone can give inhibition levels comparable to some synthetic fungicides (57). Additionally, comparable outcomes have been documented when *T. harzianum* and *Bacillus subtilis* are used to suppress *Alternaria* spp. growth and enhance the growth of mustard blight and cabbage diseases (12).

The *Trichoderma* bioagent, a talc-based formulation, has

shown superior results in controlling *Alternaria* blight disease in plants compared to other eco-friendly management approaches. The PMIL-TAN51 bioformulation treatment resulted in a minimum disease incidence of 19 % and a minimum disease severity of 18.33 %. It also reduced pod infection to 20.00 %. Biofungicides containing *Trichoderma* spp. were found to be the best seed therapy for *Alternaria* blight, with the lowest disease incidence and highest grain yield. Studies have shown that *T. harzianum* inhibits height growth and reduces disease severity in both lab and field conditions (7). According to some studies *T. harzianum* inhibited the mycelial growth of *A. brassicae* and using an antifungal preparation from *Trichoderma* led to a 20.2–29.5 % drop in the intensity of the disease (58). *Trichoderma* extracts also have significant inhibitory effects on *A. alternata* development.

Trichoderma-based products (PMIL-TAN51 bioformulation) have shown the best performance in yield contributing parameters among eco-friendly approaches. The maximum yield was observed in PMIL-TAN51 treated plots, with the maximum yield (1442.30 kg/ha) and highest number of seeds/plant (746) whereas the minimum yield (1233.33 kg/ha) and lowest number of seed/plant (395) was observed in control plots. *Trichoderma* is mycoparasitic and may produce plant growth-promoting chemicals that are volatile and nonvolatile, potentially effective against infections. Some study found that seed treatment with *Trichoderma* at 10 g/kg resulted in the lowest disease intensity, highest plant height, 1000 seeds weight and seed output. Indian mustard treatment resulted in the highest plant height, 1000 seeds weight and seed output with the lowest disease intensity (40.21 %) (9). *Trichoderma* spp. stimulates plant growth and root formation through mycoparasitism, competition and systemic resistance (59). It also exudes extracellular hydrolytic enzymes, colonises the phyllosphere and rhizosphere and generates toxic secondary metabolites (60). *Trichoderma harzianum* has the potential as a plant growth promoter due to its ability to produce hormones such as gibberellic acid, indoleacetic acid, or benzyl aminopurine, which can reduce the endo-polygalacturonase of *Alternaria alternata* by around 20 % (61). This suggests that the observed improvements in disease resistance and plant performance in *Trichoderma*-treated plants could be attributed, at least in part, to the enhanced activity of these defence-related enzymes (62). Botanical extracts were found to perform best *in vitro*, under field conditions, they were less effective than *Trichoderma*. Conversely, products derived from *Trichoderma* have shown favourable outcomes in both *in vitro* and field settings.

Conclusion

The study evaluated the antifungal and antagonistic effects of plant extracts and *Trichoderma* isolates against *Alternaria brassicicola* for disease control. Results showed that cumin seed and clove seed extracts reduced the radial mycelial growth by 93.59 %, while *Trichoderma* isolates N51, N41, HI, R1 and R50 showed the highest growth suppression (84.13 %). *Trichoderma*-based bioformulation (PMIL-TAN51) was the most effective eco-friendly treatment approach, showing minimal disease incidence, severity, pod infection and the highest yield contributions under field conditions, but botanicals performed less than that. Future research must evaluate the percentage of active substances and determine their practical form to control this disease because the

precise amounts of active ingredients in botanical extracts are unknown. As well as, in order to improve the productivity of *Trichoderma*-based products for disease management, future research should also assess their field efficacy.

Acknowledgements

The authors are grateful for the financial support by the Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh (Project NO.: 2018/348/BAU) for conducting the research.

Authors' contributions

All authors contributed to the conception and design of the manuscript. ZI and FTZ conducted the laboratory experiment and NS and AP participated in the field experiment. MAR analysed the data. The first draft of the manuscript was written by MAH, MIK and FTZ and all authors commented on the submitted version of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

- Vargas-Hernandez M, Arriaga-Madrid DA, Cortez-Perez A, Feregrino-Perez AA, Ortega-Salazar MG, Alpuche-Solis AG, et al. White mustard (*Sinapis alba* L.) residues with enhanced potential as soil biofumigant by using controlled elicitation during cultivation. *Eur J Plant Pathol*. 2023;166(2):209–18. <https://doi.org/10.1007/s10658-023-02655-6>
- Department of Agricultural Extension (DAE). Annual report on agricultural extension activities in Bangladesh. Dhaka: Department of Agricultural Extension; 2012.
- Mitrović PM, Stamenković OS, Banković-Ilić I, Djalović IG, Nježić ZB, Farooq M, et al. White mustard (*Sinapis alba* L.) oil in biodiesel production: a review. *Front Plant Sci*. 2020;11:299. <https://doi.org/10.3389/fpls.2020.00299>
- Esmaili A. Measuring competitiveness of shrimp farming in southern Iran: Using PAM approach. *World Appl Sci J*. 2008;4(5):724–9.
- Food and Agriculture Organization (FAO). Production Yearbook. Rome: FAO; 2012;57:115–33.
- Monaci E, Casucci C, De Bernardi A, Marini E, Landi L, Toscano G, et al. Brassica carinata seed meal as soil amendment and potential biofumigant. *Crops*. 2022;2(3):233–46. <https://doi.org/10.3390/crops2030017>
- Jackson KS, Kumar A. Management of *Alternaria* leaf spot on Indian mustard through chemical and biological agents. *Plant Cell Biotechnol Mol Biol*. 2019;20(3–4):162–78.
- Rahman MA, Zohura FT, Hamim I, Meah MB, Hossain MA. Sensitivity of *Alternaria* blight pathogen (*Alternaria brassicicola*) to fungicides and its effects on yield contributing parameters of mustard. *Fundam Appl Agric*. 2020;5(3):435–42. <https://doi.org/10.5455/faa.120733>
- Ravella NK, Udathala D, Iska SR. Efficacy of eco-friendly treatments on yield attributes in Indian mustard (*Brassica juncea* L.) against *Alternaria* blight. *Environ Conserv J*. 2023;24(1):171–5. <https://doi.org/10.36953/ECJ.12982369>
- Hossain MA, Habib A, Islam MS, Zohura FT, Khokon MAR. Validation of inoculation test and screening for resistance sources of mustard-rapeseed cultivars in Bangladesh against *Alternaria brassicicola*. *Curr Agric Res J*. 2018;6(1):8. <https://doi.org/10.12944/CARJ.6.1.02>
- Meena PD, Meena RL, Chattopadhyay C, Kumar A. Identification of critical stage for disease development and biocontrol of *Alternaria* blight of Indian mustard (*Brassica juncea*). *J Phytopathol*. 2004;152(4):204–9. <https://doi.org/10.1111/j.1439-0434.2004.00828.x>
- Gupta S, Didwania N, Singh D. Biological control of mustard blight caused by *Alternaria brassicae* using plant growth promoting bacteria. *Curr Plant Biol*. 2020;23:100166. <https://doi.org/10.1016/j.cpb.2020.100166>
- Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. *Agric Sci*. 2012;3(3):425–33. <https://doi.org/10.4236/as.2012.33050>
- Toppo KI, Gupta S, Karkun D, Agrawal S, Kumar A. Antimicrobial activity of *Sphagneticola trilobata* (L.) Pruski against human pathogenic bacteria and fungi. *Bioscan*. 2013;8(2):695–700.
- Mathad RC, Shakuntala NM, Vasudevan SN, Naik MN, Patil SB. Antifungal properties of aqueous extracts from *Psoralea corylifolia* Linn. seeds. *Bioscan*. 2013;8(Suppl 2):685–8.
- Singh K, Lal AA, Kumar D, Meena NK. Evaluation of selected bio agents, plant extracts and fungicides for management of *Alternaria* leaf blight of Indian mustard. *Int J Curr Microbiol Appl Sci*. 2017;6(4):26–31. <https://doi.org/10.20546/ijcmas.2017.604.004>
- Meena S, Godika S, Ghasolia RP, Sumitra, Nitharwal N, Kardam VK. Management of *Alternaria* blight disease (*Alternaria brassicae*) of mustard through plant extracts and fungicides. *Pharma Innov J*. 2022;11(1):58–67.
- Chattopadhyay C, Meena PD, Kumar SKS. Management of Sclerotinia rot of Indian mustard using ecofriendly strategies. *J Mycol Plant Pathol*. 2003;32:194–200.
- Rohilla R, Singh RL, Singh US, Singh R, Duveiller E, Singh HB. Recent advances in management of plant diseases using chemicals. *Indian J Plant Pathol*. 2001;19:1–23.
- International Seed Testing Association (ISTA). International rules for seed testing. *Seed Sci Technol*. 1996;4:3–49.
- Nasreen NE, Meah MB, Tumpa FH, Hossain MA. Effect of media composition on vegetative and reproductive growth of *Alternaria brassicicola* and *Bipolaris sorokiniana*. *Curr Agric Res J*. 2017;5(3):266–78. <https://doi.org/10.12944/CARJ.5.3.02>
- Zohura FT, Kashem MA, Hasan MA, Hossain MA. Effects of plant extracts on controlling wheat blast disease caused by *Magnaporthe oryzae* pathotype triticum. *Fundam Appl Agric*. 2018;3(2):422–33. <https://doi.org/10.5455/faa.293640>
- Salehi B, Zucca P, Orhan IE, Azzini E, Adetunji CO, Mohammed SA, et al. Allicin and health: A comprehensive review. *Trends Food Sci Technol*. 2019;86:502–16. <https://doi.org/10.1016/j.tifs.2019.03.003>
- Khan MA. Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology*. 1999;7(1):15–35. <https://doi.org/10.1007/s10787-999-0023-y>
- Zhou Y, Li C, Feng B, Chen B, Jin L, Shen Y. UPLC-ESI-MS/MS based identification and biological activities of *Allium cepa* extract. *Food Res Int*. 2020;130:108969. <https://doi.org/10.1016/j.foodres.2019.108969>
- Al-Snafi AE. The pharmacology of *Allium cepa*: A review. *Nat Prod Commun*. 2023;18(1):1–14. <https://doi.org/10.1177/1934578X231152345>
- Alqadoori SA, Dawood FA, Al-fahham AA. Active ingredients and antidiabetic activity of fenugreek: A review. *Int J Health Med Res*. 2024;3(8):594–9. <https://doi.org/10.58806/ijhmr.2024.v3i08n10>
- Moghadamtousi SZ, Goh BH, Chan CK, Shabab T, Kadir HA. Biological activities and phytochemicals of *Swietenia macrophylla*. *Molecules*. 2013;18(9):10465–83. <https://doi.org/10.3390/molecules180910465>
- Capasso R, Izzo AA, Borrelli F, Russo A, Sautebin L, Pinto A, et al. Effect of piperine on intestinal secretion in mice. *Life Sci*. 2002;71

- (19):2311–17. [https://doi.org/10.1016/S0024-3205\(02\)02019-2](https://doi.org/10.1016/S0024-3205(02)02019-2)
30. Silva MV, de Lima ACA, Silva MG, Caetano VF, de Andrade MF, da Silva RGC, et al. Clove essential oil and eugenol: A review. *Food Biosci.* 2024;62:105112. <https://doi.org/10.1016/j.fbio.2024.105112>
 31. de Souza Gomes VE, Bócoli PFJ, Iamarino JG, Grimaldi R, Ribeiro APB, Marangoni Júnior L. Neem oil: a comprehensive analysis. *Lipidology.* 2025;2(2):9. <https://doi.org/10.3390/lipidology2020009>
 32. Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on *Allamanda cathartica* using GC–MS. *Asian Pac J Trop Biomed.* 2012;2(Suppl 2):S550–4. [https://doi.org/10.1016/S2221-1691\(12\)60272-X](https://doi.org/10.1016/S2221-1691(12)60272-X)
 33. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn.: A reservoir plant for therapeutic applications. *Pharmacogn Rev.* 2010;4(7):95–105. <https://doi.org/10.4103/0973-7847.65323>
 34. Li Q, Gao WQ, Zhao YQ. Chemical constituents and biological activities of *Lawsonia inermis*. *Zhongguo Zhong Yao Za Zhi.* 2013;38(6):795–9. PMID: 23717954
 35. Zhang GY, Zeng T. Chemical constituents of *Polygonum hydropiper* Linn. *Chem Ind For Prod.* 2005;25(3):21–4.
 36. Prayogo YH, Batubara I, Sari RK, Egra S, Yamauchi K, Mitsunaga T, et al. Chemical constituents of *Acacia auriculiformis* extractives. *J Sylva Lestari.* 2023;11(3):370–81. <https://doi.org/10.23960/jsl.v11i3.753>
 37. Samanta SK, Kandimalla R, Gogoi B, Dutta KN, Choudhury P, Deb PK, et al. Phytochemical portfolio and anticancer activity of *Murraya koenigii*. *Pharmacol Res.* 2018;129:227–36. <https://doi.org/10.1016/j.phrs.2017.11.024>
 38. Beigi M, Torki-Harchegani M, Ghasemi Pirbalouti A. Essential oil composition of peppermint under drying methods. *Int J Food Prop.* 2018;21(1):267–76. <https://doi.org/10.1080/10942912.2018.1453839>
 39. Suganthi N, Dubey S. Phytochemical constituents and pharmacological activities of *Vitex negundo*. *J Chem Pharm Res.* 2016;8(2):800–7.
 40. Zoubiri S, Baaliouamer A. Chemical composition and insecticidal properties of *Lantana camara* essential oils. *J Essent Oil Res.* 2012;24(4):377–83.
 41. Dennis C, Webster J. Antagonistic properties of *Trichoderma* species. *Trans Br Mycol Soc.* 1971;57:363–9. [https://doi.org/10.1016/S0007-1536\(71\)80050-5](https://doi.org/10.1016/S0007-1536(71)80050-5)
 42. Akter T, Zohura FT, Dey S, Hamim I, Hossain MA. Effect of chemical and non-chemical management approaches in wheat blast control. *Fundam Appl Agric.* 2022;7(1):47–59. <https://doi.org/10.5455/faa.102461>
 43. Dubey RC, Kumar H, Pandey RR. Fungitoxic effect of neem extracts on *Macrophomina phaseolina*. *J Am Sci.* 2009;5:17–24.
 44. Bangladesh Agricultural Research Council (BARC). Fertilizer recommendation guide. Dhaka: BARC; 2012. 84 p.
 45. King SR. Screening and genetics of resistance to *Alternaria* diseases in Brassica. PhD thesis. Ithaca (NY): Cornell University; 1994.
 46. Kabir MH, Ara YA, Uddin AM, Islam MA, Hossain MB. Biochemical management of grey blight of mustard. *SAARC J Agric.* 2021;19(2):219–32. <https://doi.org/10.3329/sja.v19i2.57683>
 47. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. New York: John Wiley & Sons; 1984. 97–111 p.
 48. Karthikeyan R, Kumar S, Prasad R, Singh M. *In vitro* evaluation of fungicides and botanicals against *Alternaria brassicae*. *Int J Plant Pathol Microbiol.* 2021;1(1):16–9.
 49. Kumar R, Ahmed MF, Shete PP. *In vitro* evaluation of botanicals and bioagents against *Alternaria* leaf blight of mustard. *Pharma Innov J.* 2022;11(9):1–6.
 50. Suwitchayanon P, Kunasakdakul K. *In vitro* effects of clove and turmeric extracts. *J Agric Technol.* 2009;5(1):193–9.
 51. Ghasemi G, Fattahi M, Alirezalu A, Ghosta Y. Antioxidant and antifungal activities of cumin. *Food Sci Biotechnol.* 2019;28:669–77. <https://doi.org/10.1007/s10068-018-0506-y>
 52. Kavino M, Harish S, Kumar N, Saravanakumar D, Damodaran T, Soorianathasundaram K, et al. PGPR-induced resistance in banana. *Soil Biol Biochem.* 2007;39(5):1087–98. <https://doi.org/10.1016/j.soilbio.2006.11.020>
 53. Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced defense in tea plant. *Crop Prot.* 2007;26(4):556–65. <https://doi.org/10.1016/j.cropro.2006.05.007>
 54. Malathi P. Biocontrol of groundnut dry root rot. MSc thesis. Coimbatore: Tamil Nadu Agricultural University; 1996.
 55. Ghildiyal A, Pandey A. Cold tolerant *Trichoderma* strains. *Res J Microbiol.* 2008;3(8):559–64. <https://doi.org/10.3923/jm.2008.559-564>
 56. Barbosa MAG, Rehn KG, Menezes M, Mariano RLR. Antagonism of *Trichoderma* species. *Braz J Microbiol.* 2001;32:98–104. <https://doi.org/10.1590/S1517-83822001000200005>
 57. Glory YR, Rao SN, Lakshmi TV, Padma E. Management of *Alternaria* black leaf spot of cabbage. *Int J Plant Prot.* 2022;15(2):102–10.
 58. Srivastava A, Srivastava N, Sayyed RZ. Biocontrol potential of *Trichoderma* species. In: *Proc 3rd Asian Conf Plant Growth-Promoting Rhizobacteria*; 2013 Apr 21–24; Manila, Philippines. 2013. p. 432–40.
 59. Roslee NR, Doni F, Auma K, Abzar A, Yusoff WMW. Effect of *Trichoderma* spp. on mustard growth. *Undergrad Res J Biomol Sci Biotech.* 2017;1:168–76.
 60. Hossain MA, Tanjum Swarna F, Al Arabi R, Hamim I. *Trichoderma asperellum* suppresses viral diseases. *Front Agron.* 2023;5:1150359. <https://doi.org/10.3389/fagro.2023.1150359>
 61. Roco A, Pérez LM. Biocontrol activity of *Trichoderma harzianum*. *Electron J Biotechnol.* 2001;4(2):1–2. <https://doi.org/10.2225/vol4-issue2-fulltext-1>
 62. Nawar HF, Kuti JO. Phytoalexin synthesis and resistance markers in broad beans. *J Phytopathol.* 2003;151(10):564–70. <https://doi.org/10.1046/j.1439-0434.2003.00732.x>

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonpublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonpublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.